SPONTANEOUS CHROMOSOME BREAKAGE IN ASTROLOMA PINIFOLIUM.

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(Plate i; thirty-nine Text-figures.)

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Synopsis.

Astroloma pinifolium is an old and relict species with a secondary polyploid constitution. It produces pollen in variable tetrads (VPTs), which contrast with the monad pollen of diploid species of the genus. The conformity of tetrad type frequency distributions to trinomial square forms suggests a dependence upon meiotic conditions.

Meiosis in pollen mother cells is irregular. The most frequent abnormality is the occurrence of fragmentation as a consequence of chromatid breakage. Bridge configurations, which occur with a variable frequency, are due partly to chromatid reunion and partly to subchromatid errors.

A relationship must exist between chromatid breakage and the consequent loss of acentric fragments and the production of VPT pollen. A study of fragment frequency and pollen fertility in ten plants suggests that this relationship is complex.

Introduction.

Astroloma pinifolium Benth. (Epacridaceae) is a hard-wooded perennial shrub native to eastern Australia. It occurs in small local populations in the coastal region, extending from southern Queensland to eastern Tasmania. Two very isolated occurrences are in the Warialda district of north-western New South Wales and in the Grampians of western Victoria.

All plants of the species which have been examined produce variable pollen tetrads (Smith-White, 1959*a*, *b*). In plants from the coastal regions the freqencies of the five possible categories of tetrad types often conform to the terms of trinomial squares. Frequency distributions of this form suggest that the causes of VPT development may be found in the characteristics of meiosis. The two second divisions, or half mother cells, in each mother cell must have similar and independent prospects of yielding one of three possible results: both microspores derived from a half mother cell may degenerate, one only may die, or both may survive. The whole tetrad frequency distributions will then conform to the trinomial $(x+y+z)^2$.

Materials and Methods.

Investigations reported in this paper deal with the species in the east coastal region of New South Wales. The principal populations which have been studied are at Oatley and at La Perouse, both in the Sydney district and about ten miles apart. Additional populations have been examined from Pearl Beach, Tea Gardens and Evans Head, respectively, about 50, 150 and 400 miles north of Sydney. In the Grampians the species is known to be different both in its tetrad-type frequency distributions and in its meiotic characteristics, and presents a separate problem.

Acetic alcohol (1:3) and Bradley's high chloroform modification of Carnoy (Bradley, 1948) have been used as fixatives, followed by aceto-carmine and aceto-orcein stains. The chromosomes of the species stain poorly, and observations have been made with phase contrast. Feulgen staining has proved difficult owing to the characteristics of the cytoplasm, but confirmation of the presence of fragments has been obtained by its use.

Observations.

1. *The Karyotype.*

Mitosis has been examined in various tissues, including leaf initials, ovary wall, ovule integument, and embryos. It is entirely regular in these tissues, although polyploid cells have been found in young petals. At prophase, following cold treatment

at 1–2°C. for 48 hours before fixation, the chromosomes show a banded or beaded structure which is suggestive of the localization of heterochromatin (Text-figure 1), but it has not been possible to define precisely the banding pattern nor to match the pattern in homologous chromosomes. The banding may have significance in relation to the occurrence of chromosome fragmentation in the pollen mother cells.

The somatic chromosome complement $2n = 14$ (Text-figure 2; Plate i, figure 1) contrasts with a basic genome of $x = 4$ in other species of the genus (Smith-White, 1955). Centromeres are median or submedian, and four chromosome length classes can be distinguished: long (two pairs), medium long (two pairs), medium short (one pair), and short (two pairs). The ratio of the length of the longest and shortest chromosomes approaches 3:1. One pair of the longest chromosomes possesses secondary constrictions. The karyotype of other species, with $2n = 8$, is quite different, all the chromosomes being of equal length and having median centromeres. It is probable that the origin of *A. pinifolium* has involved first allopolyploidy and then structural change and the loss of a pair of centromeres.

2. *Meiosis in Pollen Mother Cells.*

(*a*) *Chiasma characteristics.*

The early stages of first prophase are unfavourable for study, and no satisfactory observations have been made of pachytene or of diplotene. At diakinesis seven bivalents are present (Plate i, figure 2), but an analysis of chiasma characteristics at this stage has proved impossible.

With rare exceptions, seven bivalents are present at first metaphase. Usually the chromosomes of each bivalent are held together by chiasmata in both arms. In the larger bivalents there may be both proximal and distal chiasmata, with a strong suggestion of chiasma localization (Text-figures 3, 4). With proximal chiasmata on both sides of the centromeres there is a marked stretching effect, and strong centromere action is apparent even in the absence of proximal chiasmata. The smaller bivalents often have one or two chiasmata in one arm only (Text-figure 4), and where proximal chiasmata are absent open ring bivalent configurations result. With only one chiasma in a bivalent, the associated chromosomes may show precocious separation (Text-figure 5), and it is difficult to distinguish this from rare occurrences of univalents (Text-figure 6). That true univalents do occur, however, is indicated by occasional misdivision (Text-figure 7). At full metaphase, univalents may lie at the extremities of the spindle (Plate i, figure 3) in a manner reminiscent of univalent behaviour in the triploid *Leucopogon juniperinus* (Smith-White, 1948).

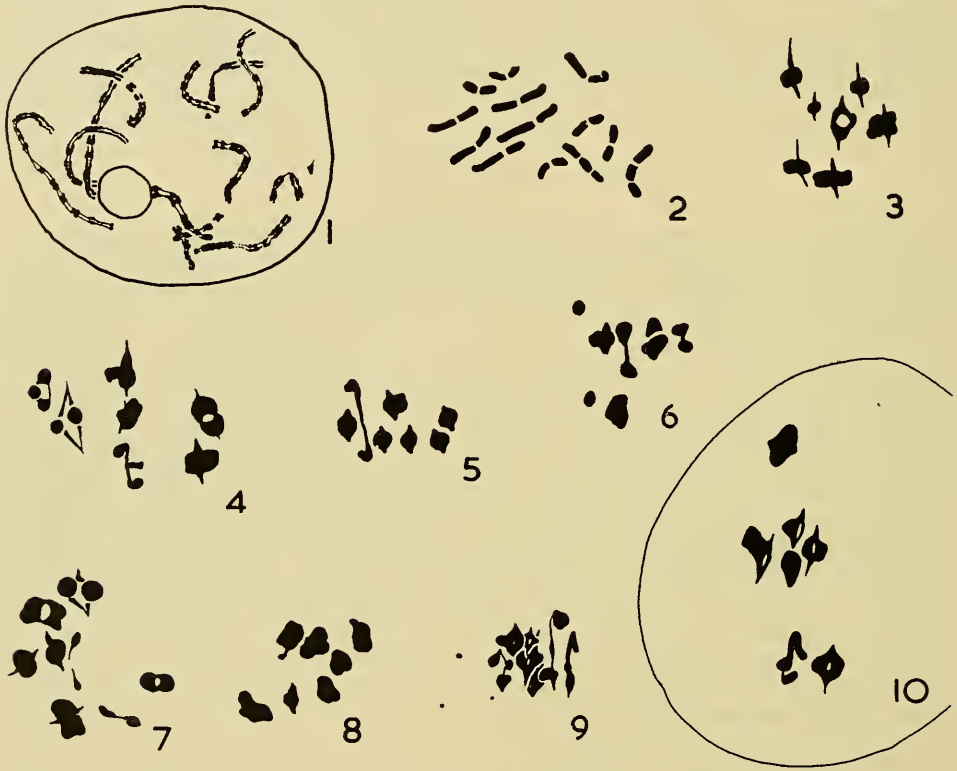
(*b*) *Abnormalities of first metaphase.*

In many plants, and possibly in all, there is heterozygosity for a small terminal knob on one chromosome (Text-figures 8, 13; Plate i, figure 4). Owing to its small size, consistent observations of its presence have not been possible, but it is present in at least ten plants of the Oatley locality.

In from 1% to 10% of mother cells in different plants the congression of bivalents to the metaphase plate may be imperfect (Text-figure 10; Plate i, figure 5). The plate may be loose, with the bivalents spread over the middle quarter or half of the spindle, or bivalents may be excluded altogether from the spindle. Such bivalents are often retarded in phase when the spindle has passed into anaphase. Text-figure 11 illustrates a particularly large mother cell in which there has been almost complete disruption of the division.

Failure of congression at metaphase must occasionally result in the loss of whole chromosomes from the daughter nuclei. However, in plant 057/5, which has an unusually high proportion (10%) of mother cells affected in this way, the exclusion of whole chromosomes from the interphase nuclei is much less frequent, and many of the misplaced chromosomes must ultimately regain their correct positions.

A third condition consistently visible at first metaphase, and throughout the first division, is a lateral displacement of the spindle. This displacement is probably present in all mother cells, but is apparent only with suitable orientations of the cells relative to the optical axis of observation. The displacement suggests the presence of cytoplasmic differentiation, and the possible relationship between cytoplasmic polarity and VPT production in *Acrotriche fasciculiflora* has been briefly considered elsewhere (Smith-White, 1959*b*). However, lateral spindle displacement is generally characteristic of other species of the Styphelieae, both in association with regular monad and regular full tetrad production, and it cannot be the immediate or direct cause of VPT production.



Text-figures 1-10.

1. Somatic prophase in ovary wall tissue. 2. Somatic metaphase, ovary wall tissue after cold starvation. 3-10. First metaphase in P.M.Cs: 3, 4. Strong centromere action and the presence of proximal and distal chiasmata are indicated. 5. A single chiasma in one bivalent allows precocious separation. 6. Two univalents, probably due to precocious separation. 7. Univalent misdivision. 8. Heterozygosity for a terminal knob. 9. Minute fragments and possible errors of chromosome splitting. 10. Three bivalents are off the spindle plate. (× *ca.* 2500.)

Other abnormal conditions which are infrequently seen at first metaphase include the presence of small or minute fragments, configurations suggesting errors in chromosome splitting (both illustrated in Text-figure 9), and neocentric activity (Text-figure 13). Neocentrics are also visible at second metaphase. Most cases of neocentrics which have been reported occur in the Gramineae (e.g., in Rye, by Praaken and Muntzing, 1942; Ostergren and Praaken, 1946; Rees, 1955; in Maize, by Rhoades and Vilkomerson, 1942, and Rhoades, 1952; in *Bromus* species hybrids by M. S. Walters, 1951, 1952, and in *Phalaris* by Hayman, 1955). However, Peacock (unpublished data) has observed strong neocentric activity at second metaphase in *Brunonia* in the family Goodeniaceae.

This and the present case are the first two examples of neocentric activity known to us outside the Gramineae.

(c) *First anaphase.*

The comparative regularity of first metaphase contrasts with the extreme irregularity of anaphase. Whilst 90% or more of the mother cells possess a normal metaphase plate, 95% or even 100% of cells at anaphase exhibit chromosome or chromatid fragments, and there is an appreciable frequency of bridges and associated abnormalities.

Fragments vary in number and size, and in arrangement in the cell. In number they range from one to as many as twelve, but numbers in excess of eight are infrequent (Text-figures 14–21). Significant differences occur in mean fragment frequency per mother cell between plants from the same population (Table 1). and there is very probably a real difference between populations, although more data would be required to establish this point.

The data in Table 1 also show that there is a positive correlation between fragment frequency and pollen death. Thus, loss of fragments must often be lethal. The correlation is not exact, however, and a simple relationship between fragmentation and pollen fertility may not operate.

TABLE 1.

The Relationship between Pollen Fertility and Fragment Frequency.

Plant.	Pollen Fertility.		Mean Fragment Frequency per Mother Cell.
	%	N.*	
O57/5	47·9	25864	1·22 ±0·15
O57/1	38·4	37696	2·96 ±0·28
O57/6	36·1	36004	1·58 ±0·16
R57/6	27·1	61136	3·35 ±0·62
O57/2	26·5	30176	2·87 ±0·28
R57/11	23·3	36392	3·74 ±0·26
R57/15	23·3	37556	2·95 ±0·18
R57/7	12·5	51012	4·44 ±0·32
LP57/4	8·3	18604	4·13 ±0·25
R57/8	7·3	38380	3·15 ±0·28

* N is the number of pollen grains on which the estimate of pollen fertility was made. It is equal to the number of tetrads scored in tetrad analysis, multiplied by 4.

Most fragments are small or minute, and spherical in shape, with a diameter equal to or less than that of chromatids. Larger fragments are rod-shaped, and may have a diameter equal to that of whole chromosomes. Rarely, large fragments may approximate in size to whole chromosome arms (Text-figure 19). It has not been possible to demonstrate any discontinuity in the range of fragment size among the smaller fragments, but there must be a much greater prospect of fragmentation near the ends of the chromosomes than near the centromeres.

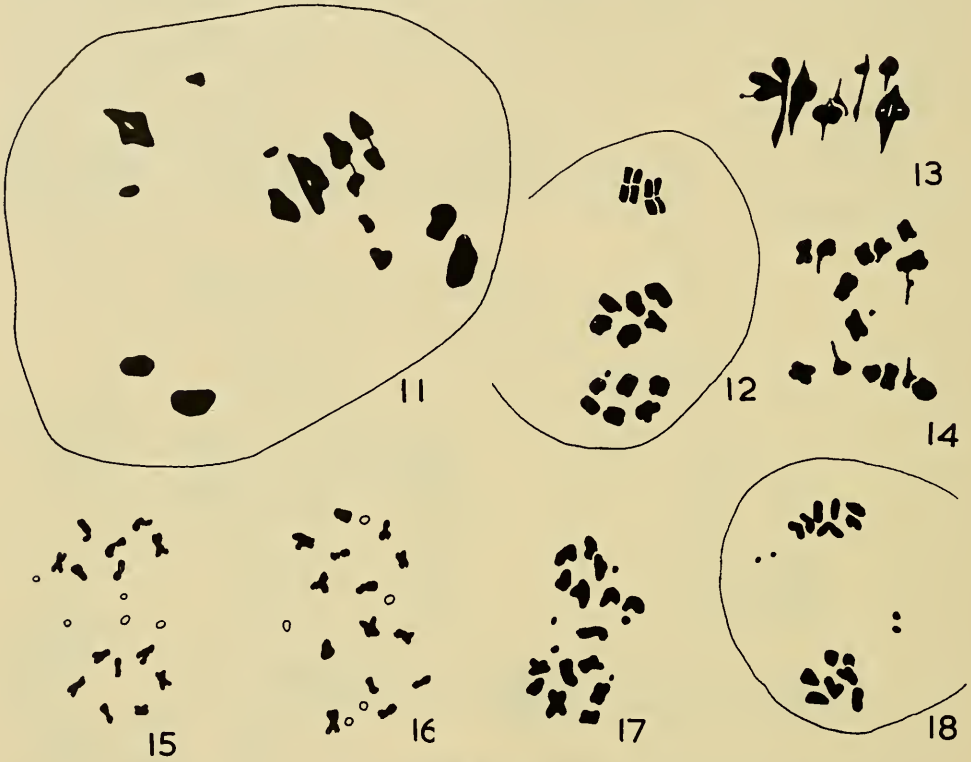
Fragments are most often single, but may be double, or they may occur in more or less closely associated pairs (Text-figures 18–20, Plate i, figures 9, 10), as though sister acentrics have separated. Such paired fragments are usually similar in size, but they may be slightly or grossly unequal (Text-figure 21).

In plant R57/15, with a mean fragment frequency of 2·95 fragments per cell, most mother cells can be classified into one of six categories having (i) no fragments, (ii) one fragment, (iii) two unpaired and unequal fragments, (iv) one pair of fragments. (v) three fragments, with two paired, and (vi) two pairs. Both larger and smaller pairs can be recognized (Text-figure 18). Although a few mother cells in this plant contain five or six fragments, there are probably two main loci susceptible to breakage.

The behaviour of the fragments during anaphase is consistent with their acentric nature. They usually lag in the mid-region of the spindle (Text-figures 15, 18, 19), and

are often at its extreme edges. With the disappearance of the spindle at telophase they are usually excluded from the interphase nuclei, and may drift to the periphery of the cell (Text-figure 33). They may sometimes be included in the anaphase groups, apparently by chance circumstances (Text-figures 16, 17).

As a result of breakage, damaged chromatids must often be present in the first anaphase and second metaphase groups. Such damage can be recognized when the anaphase chromosomes are heterozygous (Text-figures 22, 23, 24, 35), but is unrecognizable when the chromosomes are homozygous for damage.



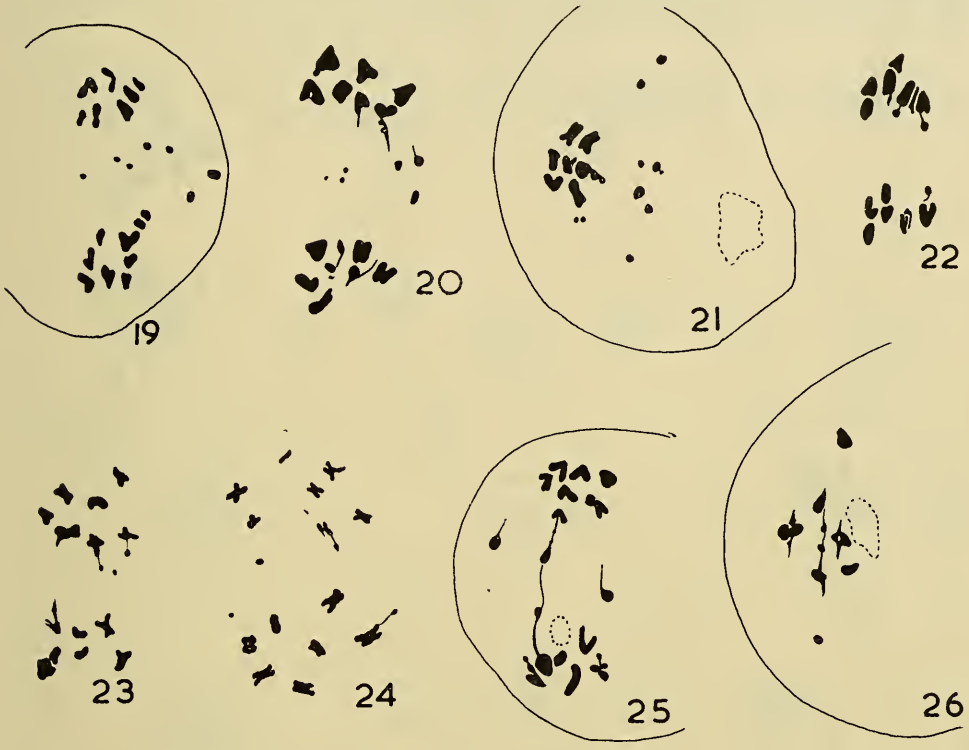
Text-figures 11-18.

11. First metaphase with extreme breakdown, in a mother cell of unusually large size. 12. First anaphase, with two chromosomes off the spindle. 13. First metaphase, showing neocentric activity and heterozygosity for a terminal knob. 14. A.1, the cell illustrated in Plate i, figure 6. 15-17. A.1, with numerous fragments. 18. A.1, with two pairs of associated fragments. (× *ca.* 2500.)

The presence of small chromatid segments which remain attached to the anaphase chromosomes by extremely fine chromatin threads (Text-figures 22–24) is obviously associated with and related to the occurrence of fragments. Following the usage of Levan and Tjio (1948), these will be referred to as "attached fragments". Most often anaphase chromosomes are heterozygous for such attached fragments, and it is occasionally possible to match homologous chromosomes at opposite poles (Text-figure 22). At other times an attached fragment at one pole can be matched with a free fragment in the spindle (Text-figure 23). Attached fragments may lag in the spindle, the connecting filament being greatly attenuated, or there may be a fine chromatin strand stretching from the attached fragment towards or across the equator of the spindle, suggesting a remnant of a chromatin bridge.

A second feature which must be associated with breakage is the occurrence of chromatin bridges. Bridges, however, are much less frequent than fragments, and

occur in less than 10% of mother cells. Types of bridge configurations are illustrated in Text-figures 25 to 30, and Plate i, figures 11 and 12. In most cases they consist of fine chromatin threads joining anaphase chromatids. They may be uniformly fine throughout their length, or apparently broken in the mid-region (Text-figures 14, 20, 27, 29), or may have one or two unstretched chromatin blocks attached at the centre (Text-figures 25, 26). Breakage may leave them with the appearance of tandem



Text-figures 19-26.

19. A.1, with large and small fragments. 20. A.1, with fragments and broken subchromatid (?) bridges. 21. A.1, with many fragments, including an unequal pair. 22. A.1, with attached chromatid fragments, two evidently being homologous. 23, 24. A.1, with free and attached fragments. 25, 26. A.1, with two-side-arm bridges probably due to subchromatid errors. (× *ca.* 2500.)

attached fragments (Text-figure 29), or there may be two "opposed" knobs stretching out from the telophase nuclei (Plate i, figure 12). Some bridge configurations are extremely complex and difficult to interpret (Text-figure 30).

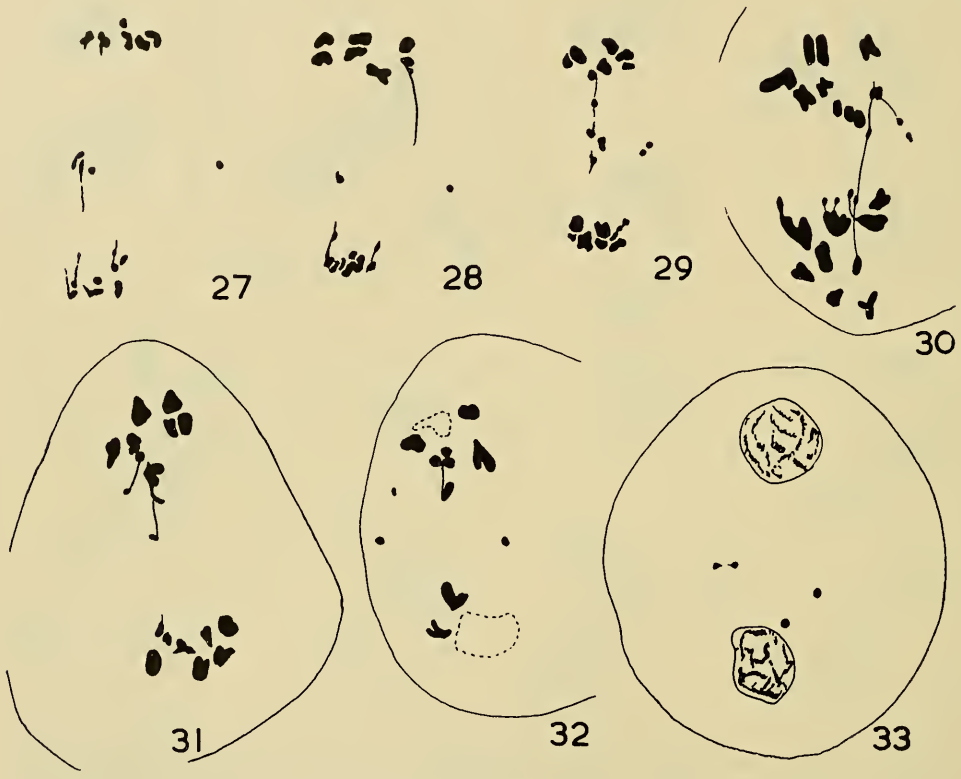
(d) Interphase and the second division.

Fragments which are excluded at telophase remain condensed throughout interphase (Text-figure 33). They may retain their arrangement in associated pairs, or may move apart. They drift to the periphery of the cell, and lie outside the second division spindles (Text-figure 34).

At second metaphase and anaphase, the following abnormalities can be recognized: (i) fragments in the spindles in addition to those in the outer cytoplasm (Text-figure 37); (ii) telocentric or near-telocentric half-chromosomes; (iii) chromosomes heterozygous for the loss of terminal segments (Text-figure 35); (iv) residual first division bridges; (v) second division dicentric and sticky bridges (Text-figure 36); (vi) neocentric activity; and (vii) misdivision (Text-figure 38). Some of the fragments which

occur within the spindles will be those which were included in the interphase nuclei, but others may be the results of additional breakage during interphase, or may be consequences of secondary breakage following reunion and bridge formation. A detailed study of fragment frequencies at first and second anaphases is needed to clarify this point.

Fragments remain condensed and visible in the cytoplasm during second telophase and the four-nucleate stage (Text-figure 39). After the formation of the microspores they degenerate.



Text-figures 27-33.

27-31. Late A.1, with remnant bridges and fragments. In 30 the bridge is complex, and there are several attached fragments. 32. A.1, showing misdivision at or close to the centromere, and several fragments. 33. Interphase, with fragments excluded from the nuclei. (× *ca.* 2500.)

Discussion.

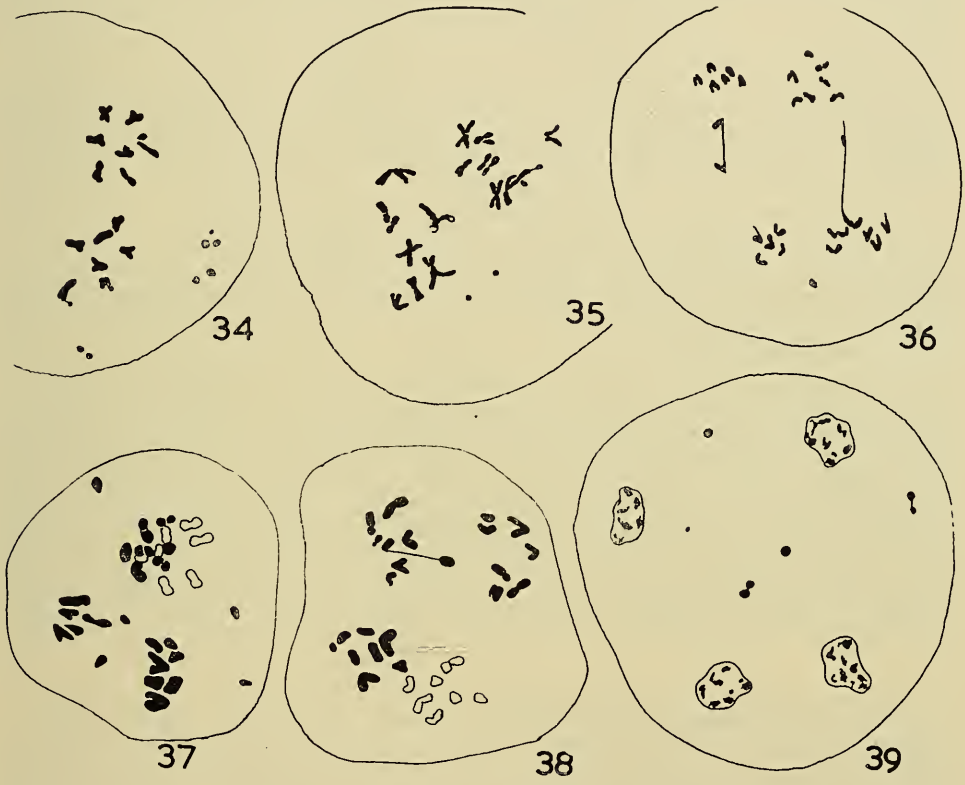
1. *Spontaneous Chromosome Breakage.*

Spontaneous chromosome breakage has been found most frequently either in temporary tissues such as anther tapetum (Cooper, 1933) and endosperm (Clark and Copeland, 1940; Brock, 1954; Rutishauser and La Cour, 1956), or in abnormal clones, wide species hybrids, or artificially inbred lines of outbreeding species.

Spontaneous meiotic breakage has been reported in horticultural clones in *Lilium* (Darlington and Upcott, 1941), *Trillium* (Sparrow and Sparrow, 1950), *Scilla* (Rees, 1952), *Paris* (Haga, 1953), *Dahlia* (Dowrick, 1953) and *Allium* (Darlington and Haque, 1955); in genetic mutants in *Zea* (Beadle, 1932, 1933; McClintock, 1950, 1951, 1953); in inbred *Secale* (Lamm, 1936; Muntzing and Akdik, 1948; Rees, 1955; Rees and Thompson, 1955); and in *Bromus* species hybrids (M. S. Walters, 1950, 1951). In none of these

cases does the material constitute part of a natural population. Marquardt (1952) and J. L. Walters (1956), however, have reported spontaneous breakage in natural populations of *Paeonia*. In *Paeonia californica* there is a high frequency of interchange hybridity, and Walters has suggested that fragmentation may be a byproduct of a system where the relatively frequent origin of new structural arrangements has been favoured by selection. Spontaneous breakage must be determined by genotypic unbalance, or by unbalance between the chromosomes and the cytoplasm. It occurs more frequently in meiosis than in mitosis, and Swanson (1958) has suggested that this may be due to a greater vulnerability in meiosis.

We are not aware of any previous records of regular chromosome breakage in a vital germ-line tissue in a sexual perennial species lacking any means of vegetative reproduction. In *Astroloma pinifolium* chromosome diminution must constitute a



Text-figures 34-39.

34, 35. M.2, showing free paired and unpaired fragments, attached fragments, and damaged chromatids. 36. A.2, with a dicentric bridge in one spindle and a sticky (subchromatid?) bridge in the other. 37. A.2, with fragments in the spindles as well as in the outer cytoplasm. 38. A.2, showing misdivision close to a centromere. 39. T.2, with single and paired (dividing?) fragments in the cytoplasm. (× *ca.* 2500.)

significant characteristic of the genetic system, and must be balanced against selection. The evolution of such a system must have involved very unusual circumstances, and these have been examined elsewhere (Smith-White, 1960, in press).

2. *The Nature and Time of Breakage.*

Darlington and Haque (1955) point out that the occurrence of free fragments at first metaphase indicates breakage before pachytene, in the premeiotic resting stage, or even in the premeiotic mitoses. Following breakage at pachytene in *Secale* and

Scilla (Rees, 1952, 1955), fragments are held in close association with their parent chromosomes by chiasmata or by coiling. In *A. pinifolium*, free fragments at first metaphase are rare. Most become free only at anaphase, and breakage during or after pachytene must be inferred. Some fragments which appear during the second division may be secondary consequences of other kinds of error, particularly from the formation of partial chiasmata (Darlington and La Cour, 1953).

In *Scilla* (Rees, 1952), *Secale* (Rees, 1955; Rees and Thompson, 1955) and *Allium* (Darlington and Haque, 1955), most, if not all, breakage is B″. In *Vicia* (McLeish, 1953), chemically induced breakage at mitosis is also B″, and is localized in regions of heterochromatin. It is almost always difficult to distinguish between whole chromosome breakage, B″, and isolocus chromatid breakage, 2B′. Darlington and La Cour (1945) and Darlington and Koller (1947) believed that all isolocus sister chromatid breaks result from presplit whole chromosome breakage. Thoday (1953), however, has shown that true isolocus chromatid breakage does occur in mitoses in *Vicia* roots following irradiation.

In *Astroloma pinifolium* most of the fragments visible at first anaphase are single, and their diameters are only equal to or less than that of normal anaphase chromatids. B′ must be inferred. Additional evidence of B′ is found in the occurrence of unequal fragment pairs and in the association of attached and free fragments (Text-figures 21, 23). Where there are paired fragments of equal size, or where homologous chromosomes at opposite spindle poles are both heterozygous for attached fragments (Text-figure 22), it is impossible to distinguish the three theoretical possibilities of B″, isolocus sister chromatid 2B′, or isolocus non-sister homologous chromatid breakage. However, with a high frequency of B′ and with localization of breakage such as is suggested by the fragment characteristics in plant R57/15 (*v. supra*), both sister and non-sister isolocus breakage is to be expected.

The occurrence of B″ or of B′ is related to the time of breakage, the former occurring before, and the latter after pachytene splitting. In *A. pinifolium* most breakage must occur later than the two-strand stage, but the appreciable frequency of isolocus breaks suggests that it may occur at or very close to the moment of splitting. It may therefore be related to errors in chiasma formation. Oehlkers (1953) denies any relationship between chemically induced breakage and chiasma formation, and this must be generally true for breakage of the B″ type. Revell (1953), however, concludes that diepoxide causes breakage at the time of chromosome splitting by a process related to normal chiasma formation. Such breakage might well be restricted to non-sister chromatids.

3. *The Interpretation of Bridge Configurations.*

The bridges which have been observed are clearly not due to inversion hybridity. Some are of simple dicentric structure (Text-figures 27, 36), and are probably due to sister chromatid breakage followed by SR, with either reductional or equational first division chromatid separation, to give first or second division bridges. They could also be due to non-sister breakage and reunion. The infrequency of fragment (i.e., distal) reunion suggests that both SR and NSR proximally may also be rare, contrary to the behaviour demonstrated by Rees and Thompson (1955) in inbred rye. Differences in the frequency of proximal and distal reunion, however, are not uncommon and, together with restriction of neocentric activity to centric chromosomes, have led Darlington (1951, 1956), Rhoades (1951) and Rees (1953) to the view that there is centromere control of both these types of behaviour.

If breakage is related to chiasma formation, some bridges may be due to partial or subchromatid crossing over. Partial chiasmata have been described in *Uvularia* (Darlington and La Cour, 1953) and in *Scilla* (Rees, 1953). Subchromatid chiasmata or subchromatid breakage and reunion could yield two-side-arm bridges similar to those figured in irradiated pollen mother cells in *Trillium* by Wilson, Sparrow and

Pond (1959). In the *Trillium* material there can be no suggestion that the subchromatid breakage is related to chiasma formation, since it can be induced by irradiation at diplotene. In *A. pinifolium* bridge configurations which are suggestive of partial chiasmata or of subchromatid breakage and reunion are illustrated in Text-figures 25 and 26 and in Plate i, figure 11. The "opposed knobs" illustrated in Plate i, figure 12, may be due to breakage of two-side-arm subchromatid bridges. It is possible that some of the bridges in *Astroloma* may be of the subchromatid pseudo-bridge types illustrated for *Trillium*, in which case they could finally separate under anaphase tension without secondary damage. Text-figures 32 and 38 illustrate cases of subchromatid splitting errors close to the centromeres, which support this interpretation.

Attached fragments at first anaphase and at second metaphase may be interpreted as fragments still held in sticky contact with their parent chromosomes, or more probably, as consequences of subchromatid breakage, as described by Levan and Tjio (1948), Kihlman (1951, 1952) and McLeish (1953).

Bridges due to partial chiasmata and to subchromatid breakage and reunion, with the exception of the pseudo-bridges illustrated by Wilson, Sparrow and Pond, and the attached fragments due to subchromatid breakage, would all lead to fragmentation in the pollen grain mitoses. Unfortunately, the cytoplasm in pollen grains of *A. pinifolium* is extremely granular, and the identification of small fragments would be virtually impossible.

4. *Meiotic Irregularities and Variable Pollen Tetrads.*

Where pollen grains remain together in tetrads at maturity, any causes of appreciable pollen death will result in the production of variable tetrads. It is perhaps surprising that, whilst many cases of partial pollen sterility are known, and tetrad pollen occurs in many families, very few examples of variable tetrads are known.

The trinomial square frequency distributions of the five tetrad categories, which is a feature of the behaviour of *A. pinifolium* (Smith-White, 1959*b*), suggests that there is a relationship between VPT production and meiosis. The loss of whole chromosomes, which is an important contributory cause of comparable behaviour in *Leschenaultia* (Martin and Peacock, 1959), is much too infrequent to be a serious cause. Chromosome breakage with the loss of chromatid fragments, on the other hand, is a constant feature of all east coastal plants which have been examined, and most of the pollen mother cells are affected. Fragment loss is obviously associated with VPT production, and a clear but probably not a simple relationship has been demonstrated between fragment frequency and pollen fertility. The nature of this relationship will be considered elsewhere (Smith-White, 1960, in press).

The diversity of kinds of irregularity which occur in the pollen mother cells of *A. pinifolium* suggests that there may be a lack of balance between the chromosomes in division and their immediate environment. There is in fact a very considerable variation in pollen mother cell size at first metaphase, which may indicate a lack of efficient control in the timing of prophase. Darlington and Haque (1955) have shown that change of timing of prophase in *Allium ascalonicum* is responsible for a syndrome of abnormalities including chromosome breakage.

Summary.

Astroloma pinifolium has a pattern of distribution which indicates a relict condition. It has a secondary polyploid genome compared with the basic chromosome number of the genus.

It produces variable tetrad pollen, in which the frequencies of the tetrad categories often conform to the terms of trinomial squares.

Meiosis in the pollen mother cells is abnormal. Failure of bivalent congression, loss and misdivision of univalents, and neocentric activities, are occasionally seen. A regular spindle eccentricity at first metaphase, which is usual in the Styphelieae, is indicative of cytoplasmic polarity.

The most significant irregularity is the presence of chromosome fragments at first anaphase and later stages. There is also a variable frequency of chromatin bridging at both meiotic divisions.

Analysis of fragment characteristics and bridge configurations suggests that (i) fragmentation is due to chromatid breakage, (ii) breakage occurs at or soon after the time of chromosome splitting in pachytene, (iii) some bridges are the result of reunion or of sister reunion, but others are due to subchromatid breakage and reunion or perhaps to errors in chiasma formation, (iv) attached fragments, which are frequent at first anaphase, are also probably the results of subchromatid errors, and may lead to undetected errors in the pollen grain mitoses.

Chromatid breakage is associated with the production of variable tetrad pollen. A comparison of fragment frequency and pollen fertility data indicates a complex rather than a simple relationship. It will be necessary to reconcile the consequences of chromosome breakage with the VPT frequency distributions which are characteristic of the species.

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EXPLANATION OF PLATE I.

1. Mitosis in leaf initial. 2-12. Meiosis in P.M.Cs: 2. Diakinesis, illustrating the lateral displacement of the nucleus. 3. M.1, with two univalents at the upper pole. 4. M.1, showing a bivalent heterozygous for a terminal knob. 5. M.1. Two bivalents and two univalents off the plate. 6. A.1, with laggard chromosomes, a fragment, and a broken bridge. 7. A.1, with one bivalent off the spindle still undivided. 8. One pole of A.1, showing a chromosome heterozygous for an attached fragment. 9. T.1, with one pair of fragments in the equator of the spindle. Other fragments are present, but are out of focus. 10. M.2, with a pair of fragments in the cytoplasm. 11. T.1, with a two-side-arm bridge The connection between the two side arms is extremely thin. Bridges of this type are probably derived from subchromatid breakage and reunion. 12. T.1, with "opposed knobs", or an incomplete bridge. This type of configuration is probably related to the subchromatid bridging illustrated in figure 11. (× *ca.* 2000.)